CASE REPORT

Chronic Granulocytic Leukemia in a Horse

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Summary

A nine year old quarter horse exhibited progressive weight loss and inappetance over a 47 day period. There was clinical evidence of pleuritis and pneumonia substantiated by leukocytosis and elevated protein in pleural fluid. Over the entire period the horse was neutropenic and had circulating abnormal immature granulocytes and low numbers of blast cells. Anemia and thrombocytopenia progressively worsened. Bone marrow examination revealed very few mature granulocytes but large numbers of immature cells of the granulocytic series and marked megaloblastic transformation of erythroid cells. These findings were consistent with chronic granulocytic leukemia.

Résumé

Leucémie granulocytaire chronique, chez un cheval

Un "Quarter horse", hongre et âgé de neuf ans, manifesta de l'inappétence et une perte de condition progressive, au cours d'une période de 47 jours. Il présenta aussi des signes cliniques de pleuro-pneumonie que confirmèrent une leucocytose et un exsudat pleural fibrineux. Durant toute cette période, le cheval afficha une neutropénie, des granulocytes anormaux et immatures, mais peu de cellules embryonnaires, dans le sang périphérique. Son anémie et sa thrombocytopénie empirèrent graduellement. L'examen de la moelle osseuse ne révéla que quelques granulocytes matures, mais plusieurs cellules immatures de la lignée des granulocytes et une transformation marquée des cellules érythroïdes en mégaloblastes. Toutes ces anomalies s'avérèrent compatibles avec une leucémie granulocytaire chronique.

Introduction

With the exception of lymphosarcoma, neoplastic diseases of the equine hemopoietic system are rare. To our knowledge the report by Lewis and Leitch (1) is the only description of granulocytic leukemia. The purpose of this report is to describe a myeloproliferative disorder in a horse. Persistent leukopenia and eventually pancytopenia were accompanied by circulating abnormal granulocytes, and a cellular marrow.

Clinical History

Veterinarians of the Ambulatory Clinic, Western College of Veterinary Medicine (WCVM) examined a nine year old quarter horse gelding because of loss of condition over a six week period. The horse was thin and listless. The body temperature was 38.2°C, pulse 44 and respiratory rate 32. A healing wound was present in the left axilla. Pulmonary auscultation revealed vesicular sounds over both lung fields and increased tracheal sounds. Penicillin therapy was instituted. Over the next ten days the horse grew weaker, pleuritic friction rubs persisted and petechiae appeared on mucous membranes. The horse was admitted to the Large Animal Clinic, WCVM for bone marrow aspiration. Following three days of hospitalization the animal was discharged at the owner's request and over the succeeding 22 days its condition deteriorated gradually. The horse was then purchased by the Department of Pathology, WCVM and readmitted to the Large Animal Clinic. Physical examination revealed a thin, debilitated horse with pale mucous membranes. Temperature, pulse and respiration rates were normal. Increased sounds were auscultated over dorsal thoracic areas.

In succeeding days the horse continued to eat but remained thin and weak occasionally requiring assistance to rise. Hemorrhage was noted at venipuncture sites. On day 47 the horse, very weak, and no longer able to eat, was euthanized and submitted to necropsy.

Clinical Pathological Results

A selection of hemograms is presented in Table I. Erythrocyte numbers gradually declined and metarubricytes were frequently present in blood smears. An unusual amount of anisocytosis was apparent and an occasional giant erythrocyte with a three to fivefold increase in diameter was found.

The most significant abnormalities were in the granulocytic series with persistent neutropenia and circulating myelocytes and metamyelocytes. Many of these cells appeared to have mature neutrophil cytoplasm but round nuclei. This apparent failure of nuclear segmentation resembled that of Pelger-Huet cells (Figure 1). Occasionally large fragments of granulocytic cell cytoplasm were seen in blood films. The cytoplasm of myelocytes and Pelger-Huet-like cells was faintly positive with peroxidase stain. Poorly differentiated hemopoietic cells were found in blood smears. These blast cells were very large with a large nucleus, one or two nucleoli, and a small amount of deeply basophilic cytoplasm. A few faint red cytoplasmic granules were visible in some of these cells. Thrombocytopenia became apparent on day 17. Most platelets were of normal size with the exception of an occasional giant platelet.

TABLE I
HEMATOLOGY OF A HORSE WITH CHRONIC GRANULOCYTIC LEUKEMIA

Day	1	10	17	33	47
RBC (10^{12}/L)	4.76	4.64	4.3	3.66	3.55
HGB g/dL	7.8	7.4	7.3	6.4	5.7
PCV %	22.7	22.3	21.6	17.7	16.2
MCV fL	47	48	49	48	45
MCH pg	16.8	16.5	17.2	17.5	15.9
MCHC g/dL	34.6	33.5	33.9	35.7	34.3
Platelets (10^9/L)	Clumped	Normal	91	54	30
WBC (10^9/L)	4.0	3.6	4.3	3.8	3.7
Neutrophils (10^9/L)	320	432	731	494	296
Band Neutrophils (10^9/L)	1800	432	817	760	518
Metamyelocytes (10^9/L)	160	216	129	304	111
Myelocytes (10^9/L)	80		387	266	185
Eosinophils (10^9/L)	40	108		76	
Lymphocytes (10^9/L)	1580	2268	2107	1900	2405
Monocytes (10^9/L)		36	86		111
Metarubricytes (10^9/L)	80	108	43		74
Protein g/dL	9.1	8.3	7.4	8.2	8.2
Fibrinogen mg/dL	600	900	200	400	600

TABLE II
PERITONEAL AND PLEURAL FLUID ANALYSES FROM A HORSE WITH CHRONIC GRANULOCYTIC LEUKEMIA

	Peritoneal Fluid		Pleural Fluid
Day	5	31	31
RBC (10^{12}/L)	0.06	1.02	0.37
Nucleated Cells (10^9/L)	6.1	4.6	33.9
Neutrophils %	8	22	34
Band Neutrophils %			
Myelocytes %	3	44	52
Macrophages %	89	31	6
Lymphocytes %		3	4
Protein g/dL	2.3	2.3	5.7
Specific Gravity	1.018	1.018	1.034

The most striking feature of a sternal marrow aspirate on day 8 was an almost complete absence of the granulocyte postmitotic maturation and storage pool. The predominant cells were medium to large with round central or eccentric nuclei and pale cytoplasm (Figure 2). These cells stained positively for peroxidase. Electron microscopy of a typical cell revealed a few dense granules typical of those present throughout granulocyte development (Figure 3) (2). Many blast cells were present resembling those observed in blood smears.

Cells of the erythroid series were primarily rubricytes and metarubricytes. Many of these had remarkable nuclear abnormalities such as fragmentation, bizarre mitotic figures and asynchronous maturation of nucleus and cytoplasm. In many cells rubricyte nuclei were present in well hemoglobinated cytoplasm (Figure 2). Megakaryocytes were present and appeared to have normal morphology. Bone marrow aspirated on day 44 and imprints made at postmortem examination were similar except for increased plasma cells and decreased megakaryocytes.

Results of pleural and peritoneal fluid analyses are recorded in Table II. Peritoneal fluid was considered normal except for the presence of immature granulocytes resembling pseudo-Pelger-Huet cells observed in the blood. Thoracic fluid revealed a marked leukocytosis, elevated protein content and many psuedo-Pelger-Huet cells. In contrast to the blood considerable numbers of mature neu-

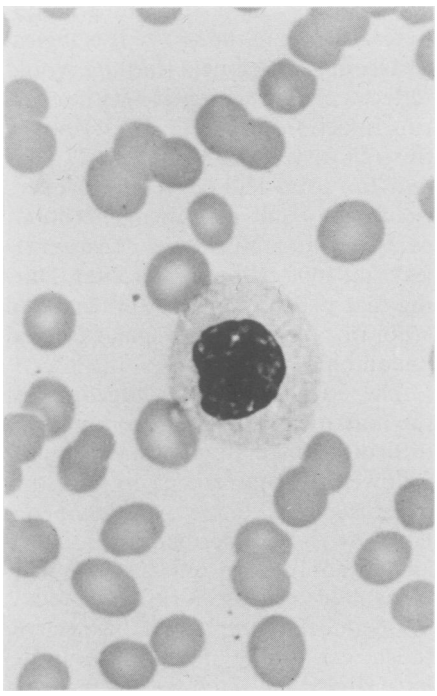

FIGURE 1. Pseudo-Pelger-Heut cell in the blood of a horse with chronic granulocytic leukemia. Wright's-Giemsa. X1250.

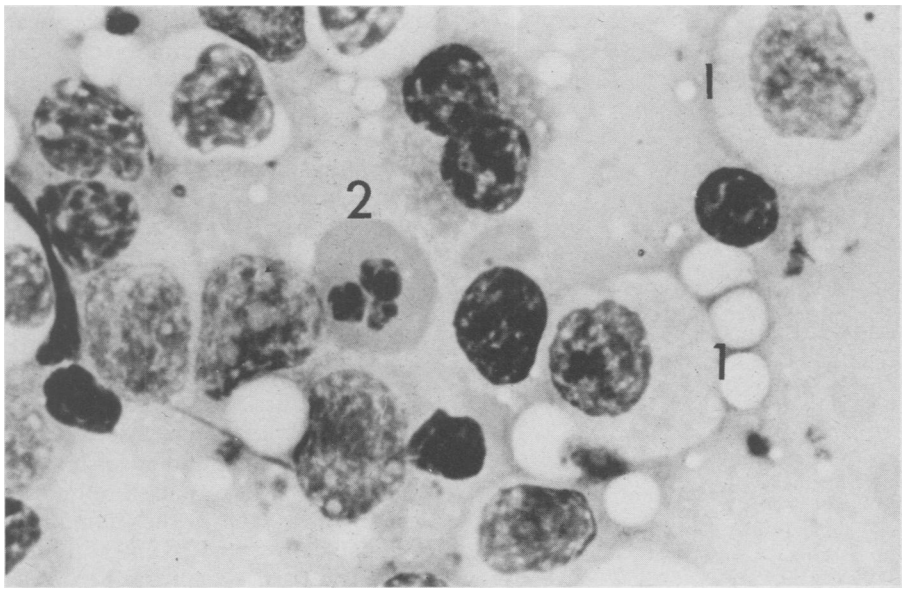

FIGURE 2. Immature cells of the granulocytic series were predominant in the bone marrow (1). A megaloblastic metarubricyte with nuclear fragmentation (2). Wright's-Giemsa. X680.

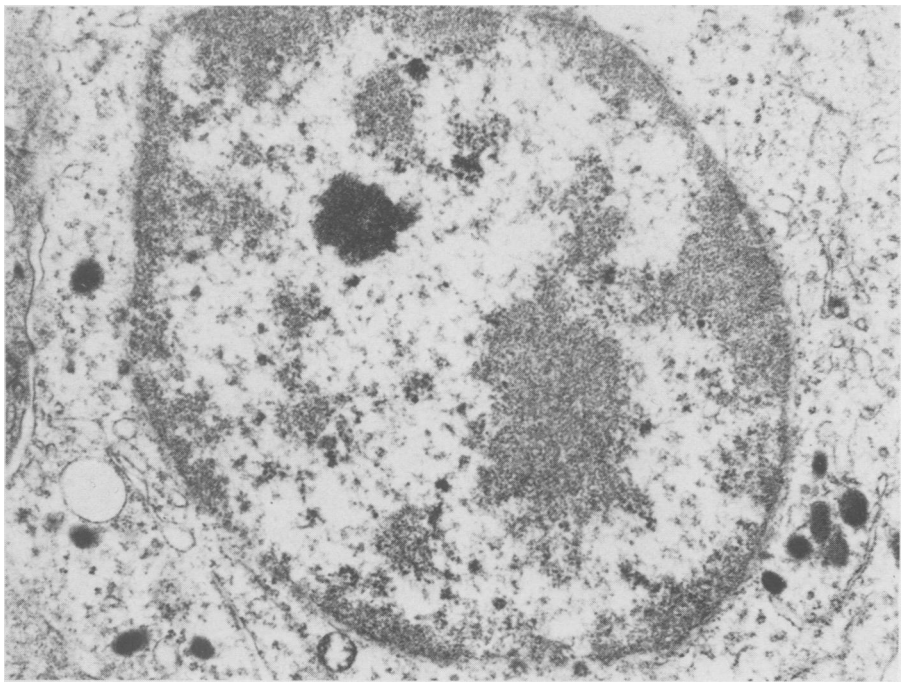

FIGURE 3. An immature cell of the granulocytic series with typical cytoplasmic granules. X7920.

trophils were present in thoracic fluid.

Clinical chemistry values are listed in Table III. Sodium, chloride, phosphorus and magnesium values were below normal. Vitamin B12 and folic acid levels were similar to those obtained from three normal horses.

Necropsy Results

The horse was emaciated. Cranial, middle and anterior caudal lung lobes were covered with fibrin, numerous adhesions and hemorrhages. Ecchymotic hemorrhages were scattered over the remainder of the visceral pleura. Numerous fibrous foci were present on the hepatic capsule and several yellow 1 cm nodules on the surface of the liver extended into the organ. The fat in the medullary cavity of the femur was irregularly replaced by red marrow, while the marrow of the ribs, sternum and ileum was uniformly red.

TABLE III
SERUM CHEMISTRY VALUES IN A HORSE WITH CHRONIC GRANULOCYTIC LEUKEMIA

Sodium (mmol/L)	129
Potassium (mmol/L)	2.4
Chloride (mmol/L)	85
Calcium (mg/dL)	9.6
Phosphorus (mg/dL)	2.3
Magnesium (mg/dL)	1.0
Urea nitrogen (mg/dL)	20
Creatinine (mg/dL)	1.3
Glucose (mg/dL)	128
Bilirubin total (mg/dL)	1.8
Alkaline phosphatase (IU/L)	105
Creatine phosphokinase (IU/dL)	6
Glutamic oxalacetic transaminase (IU/L)	143
Protein (g/dL)	8.1
Albumin (g/dL)	2.4
α 1 Globulin (g/dL)	1.4
α 2 Globulin (g/dL)	0.6
β Globulin (g/dL)	1.6
γ Globulin (g/dL)	2.1
Iron (μg/dL)	85
TIBC (μg/dL)	299
Vitamin B12 (pg/mL)	>900
Folic acid (ng/mL)	6.1

Histological examination of the lungs revealed marked fibrous thickening of the pleura. Bronchioles near the pleural surface contained in the lumen a few neutrophils and band neutrophils and many round cells with round or oval nuclei with cytoplasm resembling that of granulocytes. These were assumed to be myelocytes and pseudo-Pelger-Huet cells.

There was an occasional area of necrosis in the liver in which bacteria and fibrin were surrounded by a zone of inflammatory cells. The latter were band neutrophils and pseudo-Pelger-Huet cells. Portal fibrosis was evident accompanied by proliferation of bile ducts and varying numbers of inflammatory cells. Infiltrations of cells identical to those seen in the pulmonary bronchioles were noted in the portal connective tissues. Culture of the liver yielded Actinobacillus equuli and anaerobic streptococci.

Bone marrow sections were densely populated by a homogeneous population of cells resembling the myelocytes and pseudo-Pelger-Huet cells present in other organs. There were numerous mitotic figures. Megaloblastic transformation of red cell precursors was evident and megakaryocyte numbers appeared decreased.

Other microscopic changes were degeneration and necrosis of renal tubular epithelial cells and centrilobular hepatocytes. These changes were attributed to tissue anoxia due to anemia.

Discussion

Neutropenia may occur because of rapid neutrophil utilization or failure of granulopoiesis. The former occurs frequently in horses with conditions such as acute diffuse peritonitis and salmonellosis. Circulating granulocytes and those in the marrow postmitotic maturation and storage pool are sequestered in the area of inflammation (3). Horses will usually exhibit clinical signs consistent with these acute diseases. If the animal survives, granulocyte numbers will return to normal over succeeding days, the rate reflecting the rapidity with which the inflammation is resolved.

Neutropenia resulting from a marrow disorder and inadequate granulopoiesis is rare in the horse. It is logical to assume that clinical findings would reflect a chronic condition with recurring infections. The horse described in this report was monitored fo 47 days and the total leukocyte count never exceeded 4 300 cells per microliter. Mature neutrophils were consistently less that 1000 cells per microliter. During that period there was clinical and laboratory evidence of pleuritis and pneumonia.

The etiology and pathogenesis of the marrow disorder in this horse are difficult to understand. Marrow injury followed by neutropenia in viral diseases such as feline panleukopenia have yet to be described in the horse and in contrast to our case the neutropenia is of short duration.

Chemical compounds, especially those containing benzene radicals, have been incriminated as causing marrow injury in man (4) and less commonly in dogs (5). They usually cause injury to stem cells followed by

pancytopenia. Abnormal morphology of granulocyte precursors to the extent observed in this horse in unusual and the marrow is hypocellular.

The Pelger-Huet anomaly in leukocyte maturation occurs in man (6) and the dog (3). It is characterized by decreased segmentation of granulocyte nuclei, marked condensation of nuclear chromatin in granulocytes and normal cytoplasmic maturation (6). People with this anomaly fall into two categories, the familial and congenital or true Pelger-Huet anomaly and the acquired pseudo-Pelger-Huet anomaly (7). Despite the abnormal nuclear morphology in congenital Pelger-Huet leukocytes of people and dogs, leukocyte counts and leukocyte functions are normal. The basic abnormality in nucleic acid metabolism responsible for abnormal nuclear maturation is unknown (7).

The pseudo-Pelger-Huet anomaly in man is seen most often in chronic granulocytic leukemia (7). The distinction between the acquired anomaly and the congenital form in man rests on the absence of the anomaly in parents and the presence of diseases known to produce the acquired anomaly (7). In our horse the anomaly is presumed to be acquired because of lack of evidence for the congenital disorder in this species and the concurrent neutropenia with a cellular marrow. The persistent pleuritis and pneumonia, as well as the hepatitis apparent at necropsy with the accumulation of segmented neutrophils and pseudo-Pelger-Huet cells would suggest an inability to mount a granulocytic response capable of overcoming the infection.

The anemia in this horse was significant and became progressively more severe. The striking abnormalities, i.e. megaloblastic transformation in metarubricytes and rubricytes reflects asynchronous maturation of nucleus and cytoplasm. These changes are characteristic of vitamin B12 and folic acid deficiency (7) however their occurrence in an animal with normal levels of vitamin B12 and folic acid is difficult to explain. Perhaps it is analogous to similar abnormalities seen in feline leukemia virus induced myeloproliferative disorders where administration of vitamin B12 and folic acid fails to correct the retarded nuclear maturation (8,9). The frequent finding of megaloblastic erythroid precursors in various feline hemopoietic neoplastic diseases would suggest a similar mechanism for the dyserythropoiesis in this horse.

Thrombocytopenia became progressively more significant throughout the course of this horse's illness and reached a low of 30 000 cells per microliter. Prolonged bleeding times following venipunctures were noted at this time.

The combination of prolonged neutropenia with release of bizarre immature cells of the granulocytic series and blast cells plus concurrent dyserythropoiesis and thrombocytopenia is highly suggestive of granulocytic leukemia. The lack of a granulocyte storage pool in the marrow and the presence of large numbers of premetamyelocyte granulocyte precursors further substantiates this diagnosis. The only previously reported case of equine granulocytic leukemia is that of Lewis and Leitch (1). Their horse also became anemic and thrombocytopenic, however, megaloblastic dyserythropoiesis was not a significant finding as erythroid progenitor cells were almost absent in the marrow. Hematology revealed neutropenia and the presence of approximately 1 300 blast cells per microliter. Cells resembling Pelger-Huet cells were not observed in this case.

The primary neoplastic focus appeared to be the bone marrow in the horse reported by Lewis and Leitch (1), however there was histological evidence of neoplastic infiltration of other tissues. In our case neoplasia was confined to the marrow and myelocytes found in other tissues appeared to be the circulating cells as visualized in blood smears.

The cases of myeloproliferative diseases reported in horses, although low in number, share many of the characteristics as noted in other species. The increasing application of hematology in equine practice may reveal additional cases in the future.

Acknowledgments

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